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Prediction of delivering a small for gestational age infant and adverse perinatal outcome in women with suspected pre-eclampsia

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Short title: SGA in pre-eclampsia

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Abstract

Objective: To evaluate the test performance of 47 biomarkers and ultrasound parameters to predict subsequent delivery of an SGA infant and adverse perinatal outcome in women presenting with suspected preeclampsia.

Methods: In a prospective, multicentre observational study, 47 biomarkers and ultrasound parameters were measured in 397 women presenting with suspected preterm preeclampsia, with the objective of evaluating them as predictors of subsequent delivery of an SGA infant and adverse perinatal outcome. Factor analysis and stepwise logistic regression were performed in two pre-specified groups.

Results: In 274 women presenting at 20^{+0} to 34^{+6} weeks' gestation (Group 1), 96 (35%) delivered an SGA infant <3^{rd} customised birthweight centile (SGA-3). For prediction of SGA-3, low maternal Placental Growth Factor (PlGF) concentrations had a sensitivity of 93% (95%CI 84% to 98%) and negative predictive value (NPV) of 90% (95%CI 76% to 97%) compared to a sensitivity of 71% (95%CI 58% to 82%) and a NPV of 79% (95%CI 68% to 87%) for ultrasound parameters (estimated fetal weight or abdominal circumference <10^{th} centile). No individual biomarker evaluated had superior performance to PlGF and combinations added only small increments to test performance. Similar results were found in 123 women presenting between 35^{+0} to 36^{+6} weeks' gestation (Group 2).

Conclusions: In women presenting with suspected preterm preeclampsia, measurement of PlGF offers a useful adjunct for identifying those at high risk of delivering an SGA infant, allowing appropriate surveillance and timely intervention.

Introduction

Infants who are born small-for-gestational-age (SGA) are at increased risk of short-term neonatal morbidity(1) and mortality(2, 3), and longer term complications extending into adult life, including cardiovascular disease and type 2 diabetes mellitus(4). SGA is commonly defined as a birthweight under a centile threshold. For infants under the 10th centile for the population this group includes constitutionally small infants and those with fetal growth restriction, the latter defined as failure of a fetus to reach its full growth potential. Use of birthweight centiles customised for additional maternal (height, weight, ethnicity, parity) and fetal (sex) variables increases identification of those fetuses at risk of adverse perinatal outcomes, including stillbirth and neonatal death(5).

The underlying pathophysiology of fetal growth restriction is complex, but poor placentation plays a key role in a substantial proportion of SGA, particularly in women with preterm hypertensive disorders and when associated with adverse perinatal outcomes. There is a need for a test in the second half of pregnancy to identify those at highest risk of delivering an SGA infant. Markers of placental function could offer a useful adjunct to current methods of ultrasonography to improve risk stratification enabling identification of those at greatest risk and minimising unnecessary intervention for lower risk women. Several biomarkers have been suggested as potential predictors of fetal growth restriction, but to date, none have been shown to have adequate accuracy to support incorporation into clinical practice(6). Women with suspected hypertensive disorders of pregnancy, who present prior to 37 weeks'

gestation, are at increased risk of fetal growth restriction but the optimal strategy for identifying such fetuses remains unclear.

As part of a large prospective study in women presenting with suspected preeclampsia we sought first to evaluate 47 biomarkers (identified by an extensive literature search) and then compare the best performing biomarker(s) against currently utilised ultrasound parameters for determining subsequent delivery of an SGA infant and adverse perinatal outcome.

Methods

The PELICAN study was a prospective observational study, undertaken between January 2011 and February 2012 in seven consultant-led maternity units in the United Kingdom and Ireland. The role of placental growth factor (PlGF) in determining need for delivery within 14 days of sampling for preeclampsia in this study has previously been reported(7) and this was a planned further analysis.

Participants

Study eligibility required the presence of signs or symptoms of suspected preeclampsia in women presenting between 20^{+0} and 36^{+6} weeks' gestation with a singleton or twin pregnancy and aged ≥16 years; women with confirmed preeclampsia at enrolment were excluded. Written informed consent was obtained and baseline demographic and pregnancy-specific information were entered onto the study database. Blood was

drawn into ethylenediamine tetra-acetic acid at study enrolment and samples spun at 3000 rpm for 10 minutes. Plasma was extracted and stored at -80^{o}C until analysis. Management of the women in the study followed usual care pathways for women with suspected pre-eclampsia, as advised in the National Institute for Health and Care Excellence Hypertension in Pregnancy guidelines,(8) with ultrasound assessment being undertaken as clinically indicated.

Ultrasound assessments were undertaken by trained ultrasonographers at each study site as clinically indicated, using a variety of machined and following local protocols for measurement of fetal biometry, amniotic fluid index and umbilical artery Doppler flow velocity waveforms (as occurred in clinical practice at the time of the study). Quality control was undertaken through local procedures rather than by the research team centrally. Estimated fetal weight was calculated at each site using the Hadlock formula.(9) Additional parameters, including uterine, middle cerebral artery and ductus venosus Doppler studies were not universally reported and therefore could not be compared to biomarker performance. As study sites were reporting abnormal ultrasound assessment using a variety of parameters (including AC and EFW <10th, <5th, <3rd centiles), the most commonly reported parameters of AC or EFW <10th centile was chosen to enable comparison across sites. The presence of an abdominal circumference (AC) or estimated fetal weight (EFW) < 10th centile, oligohydramnios (amniotic fluid index < 5th centile or absent/ reversed end diastolic flow were recorded by study midwives.

Final diagnoses for maternal hypertensive disorders of pregnancy were assigned, following agreement by an adjudication panel of experts, using definitions from the American College of Obstetricians and Gynaecologists practice bulletin(10). SGA was defined as birthweight <3rd (SGA-3) customised centile (with birthweight <10th customised centile (SGA-10) as a secondary outcome), calculated using the Gestation Related Optimal Weight (GROW) method by freely available software(11). All diagnoses were assigned without knowledge of any biomarker values.

The pre-specified first part of the biomarker analysis presented here relates to two groups of women in pre-defined gestational age strata enrolled with singleton pregnancies and suspected preterm preeclampsia: Group 1 at 20^{+0} to 34^{+6} weeks' gestation and Group 2 at 35^{+0} to 36^{+6} weeks' gestation. For comparison against ultrasound parameters, the second part of the analysis was restricted to women with an ultrasound performed within 14 days of blood sampling at enrolment. The principal pre-specified outcome for both analyses was delivery of an SGA infant (defined as birthweight < 3rd customised birthweight centile)(3). The pre-specified secondary outcome measures were birthweight less than the 10th customised centile and adverse perinatal outcome. Adverse perinatal outcome was pre-defined as presence of any of the following complications: antepartum/ intrapartum fetal or neonatal death, neonatal unit admission for >48 hrs at term, intraventricular haemorrhage, periventricular leucomalacia, seizure, retinopathy of prematurity, respiratory distress syndrome, bronchopulmonary dysplasia or necrotising enterocolitis.

Biomarker measurement

The biomarkers were selected based on *a priori* knowledge of an association with preeclampsia, a biological role in placentation or a role in cellular mechanisms involved in the pathogenesis of preeclampsia e.g., angiogenesis, inflammation, coagulation. An initial panel of biomarkers was selected based on either a priori knowledge of an association with preeclampsia, a biological role in placentation or a role in cellular mechanisms involved in the pathogenesis of preeclampsia e.g., angiogenesis, inflammation, coagulation. The full list of 47 biomarkers, measured with 57 assays (where potentially biologically important assays of different epitope specificity were available) was generated following a review of the literature, appraisal of selected bibliographies and consultation with medical experts (Table S1).

Samples were labelled, and transported to the laboratory where they were spun at 3000 rotations per minute for 10 minutes. Plasma samples were tested for Placental Growth Factor (PlGF) using the Triage PlGF Test by trained laboratory staff at the study site where the sample was taken (as previously published). The additional 56 biomarker assays were analysed in a central laboratory facility (Alere, San Diego, CA) and full details of assay methods given in Text S2 and Table S3. All participants had delivered and pregnancy outcomes recorded before biomarker concentrations were analysed and revealed and all laboratory staff were masked to clinical outcomes.

Statistical analysis

Standard distributional checks showed high levels of skewness for all 57 assays, which were consistent with underlying log normal distributions. Logged values of these biomarkers were therefore used. Before considering the pregnancy outcomes, statistical factor analysis of biomarker data was undertaken, reducing the 47 biomarkers into a smaller number of highly correlated groups, solely on the basis of the correlations between the biomarkers. Factor summary scores were then calculated for all women. Consideration of scree plots and Eigen-values (> two) identified the most important factors for further analysis(12). These factors were rotated (orthogonal varimax method) so that each factor related strongly (correlation >0.6) to a small number of biomarkers only (factor analysis displayed in Table S4).

The factor scores were entered into a multiple logistic regression model for prediction of subsequent SGA. Two factors (and their biomarkers) were identified for further investigation (Tables S5 and S6). Stepwise logistic regression was used to determine which biomarkers appeared to provide additional information beyond that derived from PlGF and prediction scores were extracted for the best combinations. A comparison of Receiver Operated Curves (ROC) areas of individual biomarkers and combinations was made to see if any of the additional information was both consistent and large enough to be clinically useful. Significance was assessed through use of a non-parametric test, which allowed for non-independence of observations on the same participant, with Bonferroni correction for multiple testing.

Some biomarkers, with high uniqueness scores, were not strongly associated with any factor. To investigate whether any of these biomarkers had prognostic power in addition to that provided by PlGF and biomarkers identified earlier, stepwise logistic regression was undertaken.

The best performing biomarker was then assessed using standard test performance indices (sensitivity, specificity, predictive values and ROC areas) against currently utilised ultrasound parameters in the sub-group of women with an ultrasound scan within 14 days of blood sampling, for prediction of SGA and adverse perinatal outcome. A sensitivity analysis was conducted excluding those fetuses where the scan on the day of enrolment had abnormal findings (AC or EFW <10th centile, oligohydramnios or absent/ reversed end diastolic flow (n=20).

Statistical analysis was carried out in the statistical package Stata (version 11.2), College Station Texas, USA. Formal significance was taken at $p<0.05$. The pre-specified sample size was calculated for accurate estimation of the sensitivity (within 10%) and specificity (within 6%) of a biomarker, assumed a sensitivity of 0.90, specificity 0.90, and 95% confidence intervals (CIs, two-tailed), for determining the primary endpoint; this required 62 patients with preeclampsia and 150 women not meeting the primary endpoint. The study is reported in accordance with Strengthening the Reporting of Observational Studies in Epidemiology guidelines (Text S7)(13).

The study was approved by East London Research Ethics Committee (ref. 10/H0701/117). Participants gave informed consent and the study followed institutional guidelines.

Results

Between January 2011 and February 2012, 274 women presenting with suspected preeclampsia and a singleton pregnancy were enrolled between 20^{+0} and 34^{+6} weeks' gestation (Group 1), and 123 women between 35 and 36^{+6} weeks' gestation (Group 2) (figure 1).

For Group 1, characteristics of these women at booking and enrolment are described in Table 1. Details of maternal and neonatal outcomes are shown in Table 2. Of 274 women, 96 women (35.0%) delivered an SGA infant <3^{rd} centile (SGA-3) (of whom 90% developed pre-eclampsia) and 130 women (47.4%) delivered an SGA infant <10^{th} centile (of whom 81% developed pre-eclampsia). Adverse perinatal outcome was three times higher (39% vs. 13%) in cases complicated by SGA-3, compared to those with birthweights appropriate for gestational age. In six pregnancies a stillbirth occurred; in five of these women, the birthweight was <3^{rd} centile. In all stillbirth cases the PlGF concentration was <5^{th} centile at enrolment and predated ultrasound abnormalities by 7 to 39 days and stillbirth by 10 to 53 days.

The predictive performance of the most promising biomarkers as depicted by ROC areas are shown in Table 3; (ROC areas for all 47 biomarkers measured are given in Table S8 and individual median biomarker concentrations in women sampled prior to 35 weeks' gestation are shown in Table S9). In isolation, PlGF had the best predictive performance, with an area under the ROC curve of 0.83 to detect SGA-3 when measured under 35 weeks' gestation (sensitivity 89.7%, 81.7 to 94.9%; specificity 58.7%, 51.1 to 66.0%; positive predictive value 53.8%, 45.7% to 61.7%; negative predictive value 91.3%, 84.6 to 95.8%). Combinations of the most promising biomarkers (Table 3) showed only minimal non-significant increases in ROC areas to predict SGA-3 (from 0.83 to 0.84) and SGA-10 (from 0.78 to 0.79).

Of women enrolled prior to 35 weeks' gestation, 129 had an ultrasound with all parameters recorded within 14 days of enrolment. The test performance of ultrasound parameters and PlGF (the best performing biomarker) for determining SGA-3 and SGA-10 are shown in Table 4 and Table S10 respectively, with PlGF alone having a higher sensitivity (SGA-3 93% (CI 84% to 98%)) and negative predictive value (SGA-3 90% (CI 76% to 97%)) than any other indicator in current clinical practice. Whilst addition of PlGF to currently used ultrasound parameters (abdominal circumference or estimated fetal weight <10th centile) increased the sensitivity to detect SGA-3 (68% to 97%), addition of ultrasound parameters to PlGF measurement did not markedly enhance sensitivity (93% to 97%). Adverse perinatal outcomes (excluding small for gestation age in this definition) occurred in 22% (60 of 274 infants). In predicting composite adverse perinatal outcome, PlGF had the highest sensitivity (90%) and negative predictive value

(90%) compared to all ultrasound measurements (n=129; Table 5). In a sensitivity analysis, performance of the ultrasound and PlGF variables was similar when those with an abnormal scan on the day of enrolment were excluded from the analysis (Tables S11 and S12).

123 women were enrolled between 35^{+0} and 36^{+6} weeks' gestation (group 2) and characteristics of these women at booking, enrolment and details of maternal and neonatal outcomes are described in Tables S13 and S14. ROC areas for all 47 biomarkers measured between 35^{+0} and 36^{+6} weeks' gestation are given in Table S13. When measured in isolation, PlGF had a ROC area of 0.69 for predicting SGA-3 and 0.74 for SGA-10; addition of CPA-4 raised this to 0.77 for SGA-3 and 0.81 for SGA-10 (Table S16). Addition of other biomarkers yielded little benefit. In this group, PlGF had higher sensitivity than all other currently used ultrasound indicators in predicting SGA infants (Tables S17 and S18) and adverse perinatal outcomes (Table S19).

Discussion

Our study has demonstrated that PlGF measurement has high sensitivity and negative predictive value in the determination of subsequent delivery of an SGA infant, and in prediction of adverse perinatal outcome, in women presenting with suspected preterm preeclampsia. We evaluated SGA <3rd birthweight centile to identify a fetus more likely to be growth restricted, rather than constitutionally small. Our study would suggest that PlGF measurement has a potential role alongside ultrasound assessment

in surveillance of high-risk women with suspected preeclampsia. This is particularly pertinent in healthcare settings where women with suspected pre-eclampsia do not routinely have ultrasound performed at presentation, where integration of PlGF with current ultrasound parameters may increase detection rates for SGA. Ultrasound has an essential role in the detection of falling growth velocity, oligohydramnios or abnormal umbilical artery Doppler waveforms, which will continue to be used to stratify surveillance and time delivery appropriately. The use of PlGF for prediction of SGA relates to this high-risk group of women with suspected preeclampsia and cannot be generalised to low-risk healthy pregnant women(14).

Of 46 additional biomarker assays evaluated in isolation or combination with PlGF, there was added minimal incremental value to the predictive performance of PlGF alone and these are unlikely to be of utility in the clinical setting. It is possible that serial PlGF concentrations, with measurements closer to outcome, may further improve predictive ability while other biomarkers may only become significant closer to outcome. Placental pathology would have been a useful additional tool in assessing for fetal growth restriction but was not available in this study.

A possible source of intervention bias is that ultrasound results were revealed to clinicians whilst biomarker results were not. At the time of the study in the UK, it was not common practice to deliver for falling growth velocity alone (i.e. pre-empting delivery of an SGA infant) unless the EFW fell below the <10th centile. Adverse

perinatal outcome (excluding SGA) was chosen as a secondary outcome to evaluate performance of the variables on this additional clinically meaningful endpoint.

This study enrolled women who presented for obstetric assessment with a broad range of symptoms and signs of suspected preeclampsia, including those with underlying maternal disease. This is more informative than evaluating the tests against normal healthy pregnant women (as in a case-control study) as it is likely to more closely reflect test performance in the usual clinical setting. The multicentre nature of the study incorporating women of geographic and ethnic diversity adds to the generalisability of the results. Further strengths of the study include all final clinical diagnoses being adjudicated by a panel of medical experts and all clinical and laboratory staff being masked to biomarker results until study completion.

It is a feature of our study that the assessments (including ultrasound examination) were performed within a local healthcare setting without referral, ultrasound or management protocols being dictated centrally by the research team. It is a strength that this pragmatic approach makes it likely that the prognostic variables would have comparable performance when translated beyond the research study, with the findings directly generalisable to similar healthcare settings. However, it is a potential limitation that such an approach does not reflect assessment of ultrasound as undertaken in some healthcare systems (e.g. by a maternal-fetal medicine subspecialist).

The findings of this study relate to similar healthcare settings where same-day ultrasound assessment is not routinely undertaken for women presenting with suspected pre-eclampsia, due to national guideline recommendations or lack of availability of trained ultrasonographers. In settings where all women with suspected pre-eclampsia undergo same-day ultrasound assessment by a maternal-fetal medicine subspecialist, performance of ultrasound may be different. As we included scans performed within 14 days after blood sampling, ultrasound may have been undertaken closer to the clinical endpoint (and would therefore not have been expected to bias against ultrasound test performance).

We are not aware of any study that has compared such a wide panel of 47 biomarkers for prediction of subsequent SGA in women with suspected pre-eclampsia. Reports on the capability of PlGF to predict SGA have been conflicting. Initial small case-control studies in the first and second trimesters for prediction of SGA found no significant relationship(15-17) but subsequent larger case-control studies(18-20) and several prospective cohorts measuring PlGF in the second(21) and first trimester(22) have reported an association between low PlGF concentrations and subsequent SGA. The few small (n=21 or fewer), mainly case control studies where measurement has been undertaken in the third trimester (including at time of delivery) generally concur with our findings of low PlGF concentrations in women with subsequent SGA infants(23-26), particularly those with significant underlying placental pathology,(27) As impaired placental function underpins a substantial proportion of cases of SGA (and pre-eclampsia)(28), an angiogenic placental factor such as PlGF has biological plausibility

for prediction. A recent systematic review of 53 studies (principally of first and second trimester prediction, and with no studies of PlGF in a similar cohort to this study) investigated the value of biomarkers in the prediction of fetal growth restriction in singleton pregnancies and concluded that PlGF emerged as the most promising of the 37 biomarkers reported(6). The finding that PlGF measurements also predicted adverse perinatal outcome is supported by two other studies(29, 30) but the first evaluated PlGF measurements in the first trimester and the second reported a combined maternal and perinatal adverse outcome.

SGA has the highest population-attributable risk value (23%) for stillbirth of all pregnancy-specific disorders(31). In this study cohort five of six cases complicated by stillbirth delivered an infant with a birthweight <3rd centile. In a setting where ultrasound is not routinely performed on all women with suspected pre-eclampsia, PlGF measurement might facilitate earlier and more accurate detection of SGA associated with perinatal mortality, allowing appropriate surveillance for those at highest risk with the aim of improving outcome. Such a strategy could allow appropriate targeting of resources to at risk pregnancies with subsequent improvements in maternal and fetal outcome.

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Table 1 Characteristics of participants at booking and enrolment (grouped by subsequent infant birthweight) under 35 weeks' gestation. Values given are median (quartiles) or n (%) as appropriate

Characteristics	**Women with SGA infant <3rd centile (n= 96)**	**Women with SGA infant <10th centile (n=130)**	**Women with infant ≥ 10th centile (n=144)**
At booking:			
Age (years)	31.9 (27.2 - 36.2)	31.9 (27.4 - 36.4)	31.7 (26.3 - 35.6)
BMI (kg/m^2)	26.8 (24.1 - 31.2)	28.0 (23.9 - 32.8)	29.3 (24.7 - 34.9)
White ethnicity	63 (65.6)	87 (66.9)	92 (63.9)
Highest systolic BP (mmHg)	120 (110 - 130)	121 (110 - 130)	120 (110 - 130)
Highest diastolic BP (mmHg)	74 (65 - 81)	74 (65 - 81)	75 (68 - 82)
Smoker at booking	17 (18.5)	24 (19.2)	29 (20.4)
Quit smoking during pregnancy	10 (10.9)	14 (11.2)	19 (13.4)
Previous preeclampsia requiring delivery <34/40	15 (15.8)	18 (14.0)	12 (8.6)
Chronic hypertension	11 (11.5)	21 (16.2)	23 (16.0)
At enrolment:			
Gestational age at sampling (weeks)	31.0 (27.6 - 33.0)	31.0 (27.6 - 33.1)	31.1 (28.0 - 33.6)
New onset hypertension	60 (63)	80 (62)	65 (45)
Worsening of underlying hypertension	16 (17)	24 (19)	32 (22)
New onset of dipstick proteinuria	58 (60)	79 (61)	71 (49)
Highest systolic BP (mmHg)	147 (137 - 160)	148 (138 - 160)	141 (128 - 156)
Highest diastolic BP (mmHg)	94 (83 - 100)	94 (83 - 100)	90 (80 - 100)

Table 2 Characteristics of delivery, maternal and neonatal outcome for women presenting before 35 weeks' gestation. Values given are median (quartiles) or n (%) as appropriate

Characteristics	**Women with SGA infant <3rd centile (n = 96)**	**Women with SGA infant <10th centile (n = 130)**	**Women with infant ≥ 10th centile (n = 144)**
Onset of labour			
Spontaneous	3 (3)	7 (5)	32 (23)
Induced	29 (30)	42 (33)	64 (45)
Pre-labour caesarean section	64 (67)	80 (62)	46 (32)
Mode of delivery			
Spontaneous vaginal	15 (16)	25 (20)	45 (31)
Assisted vaginal	5 (5)	8 (6)	21 (15)
Caesarean section	75 (79)	95 (74)	78 (54)
Adverse maternal outcome*	44 (46)	61 (47)	56 (39)
Gestation at delivery (weeks)	33.8 (30.8 - 36.1)	34.4 (31.4 - 37.3)	38.1 (36 - 39.4)
Fetal death	5 (5)	5 (4)	1 (1)
Neonatal death	2 (2)	2 (2)	0 (0)
Birth weight (g)	1537 (1043 - 1910)	1660 (1200 - 2310)	3128 (2698 - 3545)
SGA <10th birthweight centile	96 (100)	130 (100)	0 (0)
SGA <3rd birthweight centile	96 (100)	96 (74)	0 (0)
SGA <1st birthweight centile	68 (71)	68 (53)	0 (0)
Adverse perinatal outcome†	37 (39)	41 (32)	19 (13)
Maternal diagnosis			
No maternal disease	0	1 (0.8)	21 (15)
Gestational hypertension	1 (1)	1 (0.8)	25 (17)
Chronic hypertension	4 (4)	12 (9)	16 (11)
Preeclampsia	86 (90)	106 (81)	59 (41)
HELLP syndrome	1 (1)	1 (0.8)	1 (0.7)
Other diagnosis	4 (4)	9 (7)	22 (16)

* Adverse maternal outcome defined as presence of any of the following complications: maternal death, eclampsia, stroke, cortical blindness or retinal detachment, hypertensive encephalopathy, systolic blood pressure ≥160mmHg, myocardial infarction, Intubation (other than for caesarean section), pulmonary oedema, platelets $<50\times10^9$/L (without transfusion), disseminated intravascular coagulation, thrombotic thrombocytopenic purpura/ haemolytic uraemic syndrome, hepatic dysfunction (alanine transaminase ≥70IU/L), hepatic haematoma or rupture, acute fatty liver of pregnancy, creatinine >150 µmol/L, renal dialysis, placental abruption, major postpartum haemorrhage, major infection.

† Adverse perinatal outcome defined as presence of any of the following complications: antepartum/ intrapartum fetal or neonatal death, neonatal unit admission for >48 hrs at term, intraventricular haemorrhage, periventricular leucomalacia, seizure, retinopathy of prematurity, respiratory distress syndrome, bronchopulmonary dysplasia or necrotising enterocolitis.

Table 3 Test performance statistics for individual biomarkers and combinations (derived from logistic regression) to predict SGA <3rd centile and <10th centile in women presenting before 35 weeks' gestation (ROC areas with 95% confidence intervals). P values are shown for comparison of a biomarker (or combination) performance vs. that for PlGF alone. [ ] low concentration of biomarker/ratio correlated to disease

Biomarkers or combinations	**SGA <3rd centile**	**SGA <10th centile**	**P value (vs PlGF alone)**
Nephrin	0.63 (0.56 - 0.70)	0.62 (0.55 - 0.69)	<0.001
[CPA-4]	0.63 (0.57 - 0.70)	0.62 (0.55 - 0.68)	<0.001
sFlt-1	0.73 (0.67 - 0.79)	0.69 (0.63 - 0.76)	<0.001
Endoglin	0.74 (0.68 - 0.80)	0.73 (0.67 - 0.79)	<0.001
[PlGF]	0.83 (0.78 - 0.88)	0.79 (0.73 - 0.84)	-
Combinations			
[PlGF/s-Flt ratio]	0.80 (0.75 - 0.85)	0.77 (0.71 - 0.82)	0.004
[PlGF/Endoglin ratio]	0.82 (0.77 - 0.86)	0.78 (0.73 - 0.83)	0.204
[PlGF], [CPA-4]	0.83 (0.78 - 0.88)	0.79 (0.74 - 0.84)	0.560
[PlGF], Nephrin	0.84 (0.79 - 0.88)	0.80 (0.74 - 0.85)	0.475
[PlGF], Nephrin, [CPA-4]	0.84 (0.79 - 0.89)	0.80 (0.74 - 0.85)	0.390

Table 4 Test performance statistics (with 95% confidence intervals) for individual indicators and in combination to predict small for gestational age (SGA) <3rd customised birthweight centile in women presenting before 35 weeks' gestation (n=129)

Indicator	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Positive likelihood ratio	Negative likelihood ratio
AC or EFW <10th centile ‡	71.2 (57.9 - 82.2)	92.5 (83.4 - 97.5)	89.4 (76.9 - 96.5)	78.5 (67.8 - 86.9)	9.5 (4.0 - 22.5)	0.31 (0.21 -0.47)
Oligohydramnios §	18.6 (9.7 - 30.9)	98.5 (92.0 – 100.0)	91.7 (61.5 - 99.8)	57.9 (48.3 - 67.1)	12.5 (1.7 - 3.9)	0.83 (0.73 - 0.94)
AREDF ||	20.3 (11.0 - 32.8)	98.5 (92.0 – 100.0)	92.3 (64.0 - 99.8)	58.4 (48.8 - 67.6)	13.6 (1.8 - 101.7)	0.81 (0.71 - 0.92)
PlGF <100 pg/ml	93.2 (83.5 - 98.1)	52.2 (39.7 - 64.6)	63.2 (52.2 - 73.3)	89.7 (75.8 - 97.1)	2.0 (1.5 - 2.5)	0.13 (0.05 - 0.34)
Combinations						
AC or EFW <10th centile or oligohydramnios or AREDF	72.9 (59.7 - 83.6)	91.0 (81.5 - 96.6)	87.8 (75.2 - 95.4)	79.2 (68.5 - 87.6)	8.1 (3.7 -17.7)	0.30 (0.19 -0.46)
AC or EFW <10th centile or PlGF <100 pg/ml	96.6 (88.3 - 99.6)	49.3 (36.8 - 61.8)	62.6 (51.9 - 72.6)	94.3 (80.8 - 99.3)	1.9 (1.5 - 2.3)	0.07 (0.02 - 0.28)

‡ Abdominal Circumference or Estimated Fetal Weight

§ Oligohydramnios defined as amniotic fluid index <5th centile for gestational age

|| Absent or Reversed End Diastolic Flow in umbilical artery Doppler flow velocity waveforms

Table 5 Test performance statistics (with 95% confidence intervals) for individual indicators and in combination to predict adverse perinatal outcome in women presenting before 35 weeks' gestation (n=129)

Indicator	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Positive likelihood ratio	Negative likelihood ratio
AC or EFW <10th centile ‡	48.7 (32.4 - 65.2)	67.8 (56.9 - 77.4)	40.4 (26.4 - 55.7)	74.7 (63.6 - 83.8)	1.5 (1.0 - 2.4)	0.76 (0.54 - 1.06)
Oligohydramnios §	12.8 (4.3 - 27.4)	92.0 (84.1 - 96.7)	41.7 (15.2 - 72.3)	70.2 (60.9 - 78.4)	1.6 (0.5 - 4.7)	0.95 (0.83 - 1.09)
AREDF ||	12.8 (4.3 - 27.4)	90.8 (82.7 - 95.9)	38.5 (13.9 - 68.4)	69.9 (60.6 -78.2)	1.4 (0.5 - 4.0)	0.96 (0.84 - 1.10)
PlGF <100 pg/ml	89.7 (75.8 - 97.1)	40.2 (29.9 - 51.3)	40.2 (29.9 - 51.3)	89.7 (75.8 - 97.1)	1.5 (1.2 -1.8)	0.25 (0.10 - 0.67)
Combinations						
AC or EFW <10th centile or oligohydramnios or AREDF	53.8 (37.2 - 69.9)	67.8 (56.9 - 77.4)	42.9 (28.8 - 57.8)	76.6 (65.6 - 85.5)	1.7 (1.1 - 2.6)	0.68 (0.47 - 0.98)
AC or EFW <10th centile or PlGF <100 pg/ml	92.3 (79.1 -98.4)	36.8 (26.7 - 47.8)	39.6 (29.5 - 50.4)	91.4 (76.9 - 98.2)	1.5 (1.2- 1.8)	0.21 (0.07 - 0.64)

‡ Abdominal Circumference or Estimated Fetal Weight

§ Oligohydramnios defined as amniotic fluid index <5th centile for gestational age

|| Absent or Reversed End Diastolic Flow in umbilical artery Doppler

Figure 1: Participant flow diagram